

results suggest that the tension through the tail domain does not play a critical role in the processive motion. The direct interaction of the two AAA rings in the motor domain may be responsible for the “mechanical gating” to sustain alternative steps of the two motor domains on MT.

#### 2608-Pos Board B578

##### **In Vitro Reconstitution of Dynamic, ER-like, Nanotubular Networks, and of Small, Tubulo-Vesicular Transport Entities by Interactions of Cytoplasmic Dynein and Spectrin with Liposomes**

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Cells contain intricate networks of membrane tubes of nanoscale dimensions, such as the endoplasmic reticulum (ER). Much smaller tubular entities, derived by extraction from donor compartments (e.g., those emerging from recycling endosomes or the trans-Golgi network) or generated by vesicle fusion (e.g., the ER-to-Golgi transport units), function in intracellular transport. Different mechanisms are thought to underlie the morphogenesis of the complex, tubular ER network, and the formation of the small tubular transport entities that travel along microtubules. Here, we show that the molecular machinery that powers retrograde vesicle motility in neurons can interact with membranes to generate these different types of tubes. We reconstituted *in vitro* elaborate networks of interconnected membrane tubes (with ER-characteristic ring closures and three-way junctions), as well as freely moving, stable tubes and tubulo-vesicular clusters, from mixtures of the minus-end motor, cytoplasmic dynein, its regulatory complex, dynactin, the anchoring protein, spectrin, and liposomes containing acidic phospholipids, in the presence of microtubules and ATP. The tubulo-vesicular clusters contained trains of spherical liposomes attached to a small tube via elastic linkers, likely maintained together through a supravascular spectrin meshwork that encompasses both the tube and the associated vesicles. Recruitment of dynein-dynactin and spectrin from the cytosol to liposomes was stimulated by phospholipase D-induced conversion of neutral phospholipids to acidic forms, and by activation of small GTPases. We conclude that similar mechanisms underlie the generation of ER-like tube networks and small tubular transport entities. Both may be generated and maintained by the action of soluble microtubule motor complexes and anchoring proteins, which bind to phospholipids, and do not require membrane proteins. Supported by March of Dimes grant 1-FY04-240 and NIH grant R01GM068596.

#### 2609-Pos Board B579

##### **Dynein Stepping Flexibility as a Mechanism for Optimal Trafficking in the Cell**

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Single molecule experiments have revealed that even under conditions of no load, backward stepping constitutes about 20 percent of cytoplasmic dynein's steps. Sideward steps are also common, and the motor's step size distribution is very broad. Such stepping flexibility might allow dynein to efficiently navigate the crowded cellular environment and avoid obstacles. However, the high speed and processivity of the motor implies strong coordination of its two heads. The idea of head coordination through a direct physical interaction seems plausible based on structural considerations, but such a mechanism raises the question of how tight coordination and stepping flexibility are simultaneously accomplished. We use physical reasoning and mathematical modeling to explore mechanisms that optimize these two opposing motor properties.

#### 2610-Pos Board B580

##### **Drag-brake Mechanism Of A Spindle Motor kinesin-5**

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In most eukaryotic systems, kinesin-5 motors are absolutely required for mitosis and meiosis, where they drive engagement and subsequent sliding apart of the antiparallel half-spindles, whilst antagonising and/or collaborating with other motors. It was previously demonstrated that the kinesin-5 motor efficiently slows down other motors such as kinesin-1 and Ncd, suggesting it can generate resistive force as well as motive force (Crevel, I.M., Alonso, M.C., and Cross, R.A. 2004. *Curr. Biol.* 14, R411-412 and Tao, L., Mogilner, A., Civelekoglu-Scholey, G., Wollman, R., Evans, J., Stahlberg, H., and Scholey, J.M. 2006. *Curr. Biol.* 16, 2293-2302). Subsequent *in vivo* work has born this out: depletion of kinesin-5 causes faster spindle extension in *Caenorhabditis elegans* (Saunders, A.M., Powers, J., Strome, S., and Saxton, W.M. 2007.

*Curr. Biol.* 17, R453-454) and causes up to 5 times longer and much more branched axons in neurons (Myers, K.A., and Baas, P.W. 2007. *J. Cell Biol.* 178, 1081-1091). The molecular mechanisms of these dual functions are poorly understood. Using mutagenesis, we show here that we can increase or decrease the drag-force component, with reciprocal effects on microtubule sliding velocity. In particular, we report a microtubule-binding-deficient mutant of *Xenopus* Eg5 with substantially reduced drag that slides microtubules 50% faster than wild type. Another mutant, in the kinesin-5 neck linker, shows increased drag together with decreased velocity. Our results suggest that whilst strongly-bound to the microtubule, Eg5 crossbridges are tuned to divide their time between force-generating states that drive microtubule sliding, and force-holding states that resist sudden or over-rapid microtubule sliding in cells.

#### 2611-Pos Board B581

##### **Millisecond Time-lapsed Monitoring of ATP Hydrolysis by Human Eg5 Kinesin: Real-time Dynamics of Conformation and Chemistry *in vitro***

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The hydrolysis of ATP is one of the key chemical reactions in life. Its mechanistic details in biological systems have been a challenge to unravel, as chemical changes in proteins during ATP hydrolysis go hand-in-hand with a series of large-scale conformational alterations. Monitoring the dynamics of chemistry and structure has been experimentally intractable, and requires application of novel methods. Herein, we present time-lapsed monitoring of an *in vitro* ATP hydrolysis reaction by a kinesin motor protein with 180-millisecond time resolution. Kinesin proteins are one of three major categories of motor proteins, capable of using ATP hydrolysis to power force generation and subsequent movement along cytoskeletal elements in cells. Our model protein system is human Eg5 (HsEg5), a Kinesin-5 motor protein participating in the spindle pole segregation during mitosis in higher eukaryotes. Truncated to its monomeric motor domain, we purified active HsEg5 and confirmed its ability to hydrolyze ATP. To monitor dynamic structural and chemical changes during ATP hydrolysis *concomitantly*, we used difference Fourier-transform infrared (FTIR) spectroscopy with HsEg5 kinesin samples, triggering initiation of the ATPase reaction by UV-photolysis of caged ATP. Interpretation of these biological data was guided by model compound data on ATP derivatives. The time-lapse data highlighted resolution of two distinct sets of conformational changes: a series of HsEg5 structural changes that precedes ATP hydrolysis and a set of structural alterations that occurs upon onset of ATP hydrolysis. Thus, we conclude that we have the first direct observation of dynamic conformational changes caused by the ATP binding in any kinesin motor protein. Secondly, the structural modifications that occur HsEg5 when ATP hydrolysis is initiated are different than those in the substrate-binding step.

#### 2612-Pos Board B582

##### **Crystal Structure of HsEg5 in Complex with S-trityl-L-cysteine**

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Eg5 is a motor kinesin involved in the formation of the bipolar mitotic spindle which is essential for the completion of mitosis. The discovery of a class of allosteric Eg5 inhibitors has raised the possibility of a novel approach for the treatment of cancer. Monastrol and S-trityl-L-cysteine (STC) are two well-characterized inhibitors of Eg5 known to prevent ADP release by the motor domain, with the latter compound being a more potent inhibitor of Eg5 than the former. We have determined the 2.5 Å resolution crystal structure of the Eg5 motor domain in complex with STC and Mg•ADP. STC interacts with Eg5 *via* a pocket formed by helices  $\alpha_2$ ,  $\alpha_3$  and loop L5, and induces conformational changes within the Eg5 motor domain similar to those seen with bound monastrol. The necklinker is positioned in the “docked” conformation seen in the monastrol-bound Eg5 structures, and the switch I and II regions also adopt conformations similar to those observed for bound monastrol. Moreover, STC contacts with Eg5 differ from those seen in monastrol-bound crystal structures. Monastrol contacts with Eg5 are largely mediated by nonpolar surfaces of the drug. During STC binding, contact between the motor domain and polar surfaces of the drug is increased in relation to that of monastrol by over 20 Å<sup>2</sup>. STC binding occludes approximately 80 Å<sup>2</sup> more of the Eg5 surface from solvent access than does monastrol. Site-directed mutagenesis and coupled biochemical assays monitoring ATP hydrolysis were used to examine whether these residues involved in polar interactions between STC and Eg5, and residues altered in solvent accessibility upon STC binding to Eg5,